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STERILIZATION OF UNMANNED PLANETARY SPACECRAFT A REPORT ON CURRENT TECHNOLOGY

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Marshall Space Flight Center, Alabama

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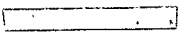

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A REPORT ON CURRENT TECHNOLOGY

by

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ABSTRACT

The present (1966) state in the rapidly expanding technology for the implementation of a planetary quarantine policy is summarized. The present status of NASA's sterilization policy and philosophy, as established by the Office of Space Science and Administration, is discussed.

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SUMMARY

The overall philosophy of spacecraft sterilization is discussed, and the present state of the art of NASA policy is presented. Biological considerations necessary for the implementation of a planetary quarantine policy are outlined, and facilities and sterilization methods are discussed.

The sterilization considerations required at each stage in the planning of a mission are discussed, and the present status of technological development in each phase of mission programming is presented, with special emphasis on problem areas and those which need further definition.

It is concluded that the successful implementation of a planetary quarantine policy is possible, but it will require maximum effort in every phase of mission planning. The greatest obstacle in obtaining this goal is seen to be the potential loss of flexibility caused by the rapid simultaneous expansion of policy and technology.

INTRODUCTION

For many reasons, including consideration of the philosophical implications of contaminating another planet, the primary objectives of planetary exploration require that planetary landers be devoid of contaminating materials which are part of the earth environment. Interplanetary investigations offer unique opportunities for obtaining information about the origin of life and its evolution which could be destroyed once and for all by the accidental introduction of viable terrestrial organisms into planetary atmospheres in which they could grow and which may contain no natural controls. It has been estimated [1] that one bacteria with a replication time of 30 days would increase to equal the bacterial population of earth in merely 8 years.

Ideally, investigation of other planets could be performed without introducing any chemical compounds at all, or at least without organic compounds, for evolutional theory is now concerned with molecular evolution. Because there exists the high probability that earth bacteria could live in and contaminate planetary environments [1] and because there is a lack of knowledge and experimental technology to study possible life forms or other contamination mechanisms smaller than bacteria (e.g. viruses), an international planetary quarantine policy which requires that planetary landers be sterile with respect to viable bacterial species has been adopted.

The development and implementation of a sterilization policy is of high priority in planning interplanetary missions. This effort involves the development of specific requirements and of techniques for their implementation.

The purpose of this report is to examine the present status of policies and requirements, and to summarize briefly the technological status of the sterilization aspect of interplanetary missions currently in the developmental stages. Particular emphasis will be placed on the problem areas which need further definition.

STERILIZATION REQUIREMENTS FOR PLANETARY EXPLORATION

The theoretical requirement for sterility implies that there are absolutely no viable microorganisms on or in a planetary lander, since this is the only stable state with respect to biological contamination. However, methods of sampling and analysis require that this be expressed as a low but finite probability that a planet will be contaminated as a result of a space mission. Because of the difficulties of analysis and the nature of the property being measured, sterility assurance must be based not entirely on physical measurements, but on a concept of certified sterility. This is the definition of a sufficient set of principles which, if followed, give an acceptable level of assurance that the level and type of contamination does not exceed a certain value. Implementation of this principle demands consideration of sterility in planning, engineering and fabrication throughout all phases of the mission.

Current NASA policy for carrying out these requirements is as follows [2]:

1. "Lander will be assembled in clean rooms at specified levels of assembly.

2. "Landing assembly will be subjected to an approved sterilization procedure.

3. "The landing assembly will be enclosed in a bacteriological barrier to maintain cleanliness and sterility. After [sterilization] the enclosure will not be opened within any portion of the earth's atmosphere which might re-contaminate the landing assembly."

Although the original maximum permitted probability of an organism surviving terminal sterilization was 10^{-4}, refinement of the analytical methods is continually being done. For example, the inclusion of a term other than unity (10^{-3} to 10^{-2}) for the probability of a lone survivor being released on the planetary surface and spreading has allowed an increase of maximum lander contamination to 10^{-3} [3]. The details of the mathematics involved in arriving at allowable contamination probabilities can be found in Sagan and Coleman, Schalhowsky and Light [4, 5, 6].

The following probabilities for the various mission phases have been proposed by Jet Propulsion Laboratory and are under consideration by NASA [7]:

"On any unmanned flight of a vehicle to the planet Mars, be it a lander, orbiter, flyby or some combination of these, the following must be fulfilled:

1. "The probability of an accidental planetary impact of any highly contamination part thereof shall not exceed 3×10^{-5} per flight. For the purpose of this constraint, 'highly contaminated' shall refer to any vehicle or part thereof which carries more than 10^2 viable organisms.

2. " The probability of the release of a viable organism on the planetary surface or in the planetary atmosphere from all other sources of contamination shall not exceed 1×10^{-3} per flight.

3. "For any source of contamination that continues to act over long periods of time, such as an unsterile orbiter, the above constraints shall apply to all potential contaminations up to and including the year 2000.

4. "The numerical constraints shall be sub-allocated to the individual contamination sources by the agency or agencies responsible for the mission design and operation."

"Using these constraints and a requirement of 99.9 percent confidence that the unmanned exploration of Mars will not contaminate the planet with terrestrial organisms, calculations have yielded an overall probability of contamination value of 1.015×10^{-3} for the unmanned program."

The planning of the Voyager mission around the outline of clean assembly, terminal sterilization by heat and enclosure in a barrier which then remains sealed, is well underway and will be covered in this report. It should be emphasized that the outline itself is subject to cricitism, however, and it is susceptable to alteration or replacement as technology and experience are gained.

One example of a possible alternative to this outline is the suggestion by Fisher [8] that terminal inflight sterilization inside a lightweight deployable canister is feasible. The advantages of this approach, such as the elimination of elaborate ground facilities for sterility maintenance after sterilization and the long flight time available for a sterilization cycle, make this a concept worth consideration.

Schalkowsky discusses the approximate values used in calculating the probabilities of contamination [5]. He suggests that with different but equally valid approximations the required contamination probability could be achieved with a small increase in terminal sterilization, without the need of a low presterilization bioload, and thus it may be possible to eliminate the costly use of clean assembly methods. This criticism is presented more as an illustration of the methods and approximations involved and the need of further evaluation than as a specific suggestion. Present development of sterilization technology is toward increasingly low presterilization bioload, and continued reduction in heat soak duration.

BIOLOGICAL CONSIDERATIONS

Introduction

The bacteria are the smallest living organisms (0.2 to 10.0 microns) about which much knowledge has been accumulated. Viruses are smaller, but little is known about the conditions which govern their ability to live in their hosts, although it is thought that they are not autonomous. For that matter, it is possible that there are other smaller "life" forms as yet unknown to man. Thus, the most resistant bacterial forms, the mesophilic spore formers, have been chosen as criteria sterility assurance.

To best explain the death of bacterial populations under exposure to lethal conditions is to say that it is governed by an exponential law, i.e., a constant time interval results in the reduction of the population by a constant fraction. The term "D-Value" refers to the time required for a 90 percent population reduction, and "Z-Value" refers to the slope of the die-off curve. The D-Value is characteristic of the organism, the sterilizing condition and the environment of the organism. The exponential law implies theoretically that zero can never be reached; thus a calculated population of less than one, e.g. 10^{-4}, is expressed as the probability of one organism surviving. Figure 1 shows an extrapolated plot of population expressed exponentially versus time on a linear scale [9].

The deviation shown represents the difficulty in enumerating at low levels, and has contributed to criticism of the plot itself. The criticism has been made that since the problem involved is that of defining the chances of the last spore surviving, and this plot depends on a random die-off and a random distribution of physiological states and resistances, a better approximation could be made using extreme-value statistical analysis [10]. Current research for the most part supports the validity of the exponential representation, and it will be used until another method is shown to be better.

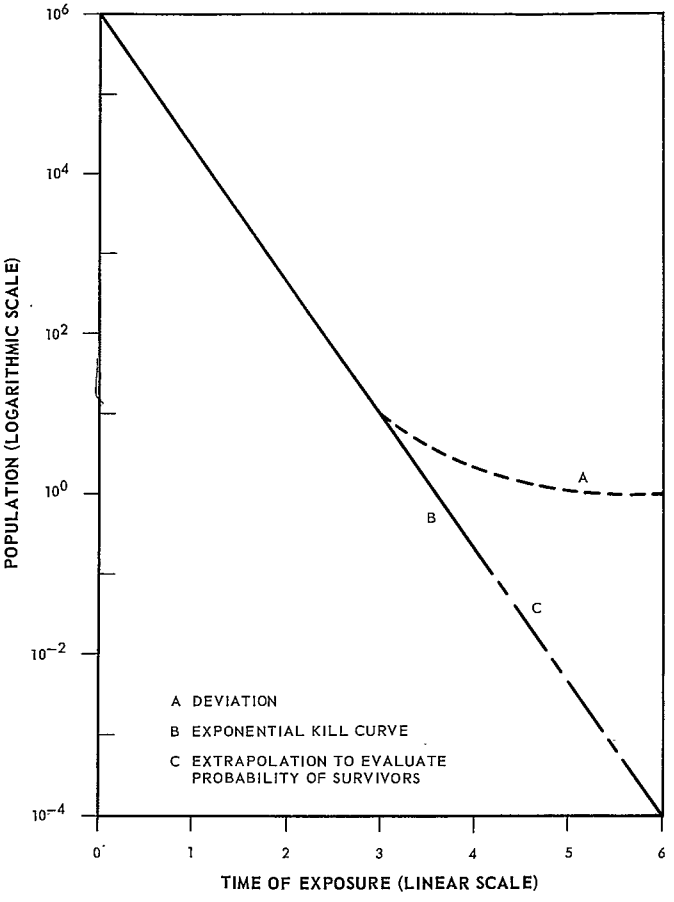


FIGURE 1. ACTUAL AND THEORETICAL ORDERS OF DEATH OF A BACTERIAL POPULATION

Sterilization Methods

1. Physical. - To meet the requirement of sterility, the internal portions of components and materials as well as the surfaces must be sterile. This requires a sterilizing agent which penetrates to the interiors of materials.

Gamma radiation is effective in penetrating and killing ability, but it is expensive, dangerous and more destructive to materials than heat [1]. However, the effects of radiation are of a different nature than those of heat, and Barrett and Cooley [11] report that failure mechanisms activated by radiation are different from those acting during a flight mission; thus radiation sterilization could possibly give higher mission reliability than heat. Another advantage would be the elimination of the weight penalty imposed in heat sterilization due to gas pressure, etc. Barrett and Cooley [11], in a study of the feasibility of using radiation for sterilization, report that sterilization by radiation is feasible and that cost is the only major disadvantage compared to heat. Further knowledge of doses, kill rates and material effects, and the development of application methods and radiation resistant components and materials will be necessary before this can become a useful tool. The combined use of heat and radiation may prove to be advantageous also.

Isolation of a part will allow many organisms to die off, if no nutrient is present. This requires a long time, however, and some materials can be nutrients to bacteria; thus, this procedure is used only as a supplement to other decontamination methods. It has been shown [12] that vacuum conditions such as are found in space are not lethal to spores.

Another possibility is the use of ultrasonics, but very little work has been done in this area to date.

Dry heat is the best and most understood method, and the only one currently approved by NASA. It has the advantages of penetration and easy measurement, as well as relatively large accumulation of death-rate information. The main disadvantages of heat include its degradation of components and materials and the stresses caused by thermal gradients and increased pressures during heating. Because of these factors, sterilization cycles are calculated on the basis of only one heating. This presents additional problems, but it is felt that these are not insurmountable. The approach

being taken is one of developing components and materials that are able to withstand sterilization temperatures, and studies are underway to devise methods to protect presterilized heat-sensitive components during the heat soak [13].

2. Chemical. - Chemical methods are only good for surface decontamination and are used for the reduction of the bioload before terminal sterilization.

Liquid decontaminants include phenol, lysol, quaternary compounds, hypochlorite, caustic sodium hydroxide and formalin; 5 percent ethylene oxide in methanol is reported to be an especially good liquid sterilant [14]. In general, liquid decontaminants are rather undependable because their effectiveness depends on the conditions and techniques of application, for example, temperature, time and pH.

Gaseous chemical sterilants are generally more effective, although many gaseous as well as liquid decontaminants are corrosive to materials. Among those that have been considered are formaldehyde, beta-propiolactone, peracetic acid, methyl bromide, propylene oxide and ethylene oxide.

Ethylene oxide has been found to be quite useful. It is explosive and highly toxic, but in the mixture 12 percent ethylene oxide and 88 percent Freon-12 (CF_2Cl_2) by weight (ETO-F12), it is nonexplosive, is effective at room temperatures and low humidities, has high penetrating powers and relatively low toxicity, is not corrosive to most materials, leaves no residue and is easily purged. The ETO-F12 mixture is an efficient sterilant or decontaminant and has been chosen as the main chemical method to be used for surface decontamination. Its main disadvantage is that it requires a relatively long time period. Beta-propiolactone is less penetrating, has a higher toxicity and may be carcinogenic; however, it requires less time and may be used in special cases when ETO-F12 concentrations cannot be maintained [15]. Propylene oxide is slower acting than ETO-F12, but less toxic; methyl bromide is only one tenth as effective as ETO-F12; formaldehyde leaves a messy residue [16].

The Russians reported at the 1966 COSPAR meeting in Vienna that they use a mixture of 60 percent ethylene oxide and 40 percent methyl bromide by volume for terminal sterilization. (They accomplish internal sterilization by sterilizing by heat all components at some stage in the assembly.)

They report that this mixture has high penetrating power and does not alter the properties of plastics or rubber or influence the functioning of radios or electric components [17]. This mixture will probably be re-examined in this country. (Preliminary plans for an international conference on sterilization in London July 1967 has been endorsed by COSPAR and NASA officials).

3. Filtration. - Filters that are capable of screening out microbial-sized particles have been developed. Decker and Buchanan [18] report an evaluation made of various types of filters using bacterial particles 1 to 5 microns in diameter, in which the filters were placed in classes according to efficiency. The highest efficiency was 99.999999 percent. This study was oriented toward air filtration but they state that satisfactory filtration of liquids can be achieved. The Russians reported sterilizing liquids by filtering through asbestos filters [17]. Further investigation of filters to be considered for sterilization is being done by the Wilmot Castle Company under Contract to Jet Propulsion Laboratory [19].

4. Use of Sporicidal Materials. - Various polymeric materials and fuels have been and are being investigated for toxicity [19, 20, 21]. Some of the problems presented by Opfell and Bandaruk [20] in the assurance of internal sterility (see discussion of sampling method) may challenge some of the findings of these reports. The approach of producing self-sterilizing materials is a good one, however, especially for materials which are overly degraded by heat sterilization. Further development in this area appears to be warranted.

5. Current Status of Approved Sterilization and Decontamination Methods. - The only NASA approved means of terminal sterilization is the use of dry heat. The first twelve cycles listed in Table I are those approved by NASA on the basis of the "worst case" approximations of an initial bioload of 10^6 viable organisms (mesophilic bacterial spores in soil), undergoing a twelve log reduction to 10^{-4} organisms [22]. The last cycle listed is a recently approved [23] cycle for Voyager which is the result of refinements in the methods and approximations used. This D-Value is based on spores embedded in plastic, and the length of the cycle is based on an initial population of 10^5 spores to be reduced to 10^{-3}. This initial population assumption is the result of improved techniques of achieving and estimating the feasible presterilization bioload [3]. Further reductions as the result of continuing research on D-Values and Z-Values are expected. NASA approved heat decontamination cycles are given in Tables II and III.

There is hope that Ethylene Oxide-Freon F12 will be authorized for limited sterilization use at some future time. It is currently approved for

TABLE I. APPROVED STERILIZATION CYCLES

Temperature (° C)	D-Values (Hours)	Sterilization Time (hours)
105	28.0	336
110	17.5	210
115	11.0	132
120	7.0	84
125	4.4	53
130	2.8	34
135	1.8	22
140	1.1	14
145	0.73	9
150	0.46	6
155	0.31	4
160	0.21	3

(Based on heterogenous mesophilic bacterial spores in soil, initial population = 10^8, final population = 10^{-4} [22]).

125	3.5	24.5

(New cycle based on spores in plastic, initial population 10^5, final population 10^{-3} [23]).

TABLE II. DECONTAMINATION HEAT CYCLES FOR ASSAYABLE ITEMS

Temperature (°C)	D-Value (hours)*	Minimum Time (hours)
100	30.0	120.0
110	12.0	48.0
120	4.5	18.0
130	1.7	7.0
135	1.0	4.0
145	0.38	1.5
155	0.14	0.6
160	0.09	0.4

* Based on thermal death-time curve (with extrapolations) for spores of Bacillus subtilis var. niger embedded in plastics.

surface decontamination before sterilization. Ethylene Oxide-Freon F12 surface decontamination consists of not less than 72 hours exposure to 35 percent relative humidity, followed exposure to not less than 300 milligrams per liter ethylene oxide for not less than 4 hours at 21° C (70° F) [22].

Radiation sterilization approval may be granted in special cases by the Planetary Quarantine Office and the Director of Lunar and Planetary Programs. Applications for this approval must include proof of need for radiation sterilization, proof that its use will not result in contamination of the entire system and proof that spacecraft reliability will not be compromised [22].

Insertion of sterilized parts and components will not be permitted unless it can be proved that the specific procedure has a probability of contaminating the spacecraft equal to or less than 10^{-4} [22]. The main problem here is the lack of sufficient technology to develop a reliable sterile insertion procedure.

TABLE III. DECONTAMINATION HEAT CYCLES FOR NONASSAYABLE ITEMS

Temperature (°C)	D-Value* (hours)	Minimum Time (hours)
105	28.0	280.0
110	17.5	175.0
115	11.0	110.0
120	7.0	70.0
125	4.4	44.0
130	2.8	28.0
135	1.8	18.0
140	1.1	11.0
145	0.73	7.3
150	0.46	4.6
155	0.31	3.1
160	0.21	2.1

* Based on spores in soil.

Sampling Methods

1. Culturing. - The best method of detecting biological contamination is culturing; other methods do not distinguish between living and dead cellular material. NASA has recently (1 June 1966) published a document [24] which covers present sampling requirements and detailed methods. A list of procedure titles is as follows:

a. Assessment of the Microbial Contamination of the Intramural Environment of Space Hardware Assembly, Test, and Launch Facilities.

b. Assessment of Microbial Contamination on and within Space Hardware.

c. Assessment of Microbial Contamination Deposited on Space Hardware Handled by Technical Personnel.

General Electric has been contracted by NASA to issue "Procedures Manual for Planetary Spacecraft to be Sterilized by Heating, Volume III, Biological Handbook," which will familiarize engineers and other technical personnel with biological concepts [14, 25].

Culturing, although it is the best method of sampling, has limitations. Incubation requires time and it only measures reproducing organisms. There is no absolute certainty that an organism in its preferred medium will reproduce. Culturing does not distinguish between dormant and dead organisms, and it is not known under what conditions a dormant microorganism will produce again. Also, there is always the danger that organisms are killed during the sampling process, or that extraneous contamination is introduced.

The recovery of microorganisms from surfaces for culturing is accomplished by swabbing or Rodac plating, which are standard microbiological techniques; the recovery of organisms from solids is more difficult and has the additional disadvantage that materials must be destroyed to be tested. It has been reported by Opfell and Bandaruk [20] that test sensitivity for solids is more dependent on the surface and material being tested than other single parameter. In some solids they were unable to detect innoculums of as many as 10^6 spores per cubic centimeter. Recovery of microorganisms from solid materials is accomplished by slicing, pulverizing [24], or dissolving. The recovery

depends in part on the method used . Each of these methods may damage or poison certain microorganisms. Bacteriostasis, which would be interpreted by present methods as sterility, can result from contact of media with tested material, contact of the organisms with tested material or preservatives in the material (e. g. , in polymeric materials).

Further development of specified [24] and alternative culturing procedures, especially for solids, will be necessary to facilitate biological monitoring and make it more dependable. Techniques must be approved and refined so that tests will apply to all potentially viable organisms, rather than just the uninjured, nondormant and easily removable ones. Appropriate media for all possible contaminants need further definition. Developmental work in the detection of contamination in solids has been and is being done by the Dynamic Science Corporation under contract to Jet Propulsion Laboratory [19].

For future developments it is suggested in Opfell and Bandaruk [20] that automated techniques for searching solid materials may be possible using life detection techniques developed for planetary missions. These rather insensitive tests could be made more sensitive by utilizing the extensive knowledge of the earth environment as opposed to the postulated Martian environment. Another possibility is the detection of injected fluorescent antibodies corresponding to the particular test organism.

The current state of technology in biological sampling of materials does not justify interpreting negative tests as meaningful, and, fortunately, sterility assurance is based on other factors as well. Sampling of the processes by which components are manufactured, packaged and sterilized is more reliable than sampling of components themselves. For complete sterility assurance it is necessary to study the complete manufacturing history of components and materials.

2. Sterility Indicators. - Another basic technique for sterility testing is the use of indicators during the sterilization process. These include temperature indicators for heat sterilization, isotope counters for radiation sterilization and chemical indicators to measure ETO concentrations. A summary of sterility indicators is given in Table IV [14]. The best and most direct method, however, is the use of spore strips. These are hermetically sealed tablets which contain a specified spore population greater than that on the lander. After the sterilization process, these are broken open and cultured. The Wilmot Castle Company under contract ot Jet Propulsion Laboratory is working on developing improved and standardized indicators [19].

TABLE IV. STERILITY INDICATORS

Sterilization Method	Indicator	Property Being Measured
Dry or Moist Heat	Thermometer Thermocouple Temperature Sensitive Paints, Labels, Crayons, Ampules	Temperature
	Bacterial Spore Strips, Ampules, Packets	Viability
ETO-F12	Chemical Indicators	ETO-F12 Concentration
	Spore Strips	Viability

Bioclean Facilities

1. Introduction. - Bioclean facilities may be defined as enclosed areas employing control over viable and nonviable particulate matter in air with temperature, humidity and pressure control as required. This includes both glove boxes and bioclean rooms. Several studies and experiments have been done on the use of both. The following paragraphs provide brief summaries and descriptions accompanied by the most current and comprehensive references to detailed information.

2. Glove Boxes. - Glove Boxes are small enclosures in which operations are performed by workers who are out side the enclosure and do their work with gloves attached to the walls of the enclosure. Studies of these with respect to cost and load reduction efficiency have been done by Lockheed [26] and General Electric [27].

3. Bioclean Rooms.

a. Specifications. A new document, "Standard Bioclean Room and Work Station Requirements for the Microbiologically Controlled Environment" [28], will replace the 1963 NASA "Interim Requirements for Bioclean Facilities." Table V shows the requirements from this document. NASA sterilization

TABLE V. BIOCLEAN ROOM REQUIREMENTS

Class	Maximum Number of Particles per Liter (per cubic foot)	Maximum Number of Viable Particles per Liter (per cubic foot)	Maximum Number of Particles 5 Micron and Larger per liter per cubic foot)
100	3.5 (100)	0.0035 (0.1)	
10 000	350 (10 000)	0.35 (10)	2.3 (65)
100 000	3500 (100 000)	3.5 (100)	25 (700)

guidelines now specify the use of class 100 laminar downflow clean rooms according to Fed. Std.209 [29] for control of biological contamination on planetary landers [22].

Although there is a close connection between particulate and viable contamination, they are not necessarily synonymous. Investigations are underway to determine what measures are necessary to obtain desired levels of microbial contamination. It is possible that methods other than, and less expensive than, laminar downflow will be found adequate to meet microbiological requirement.

For example, General Electric investigated several flexible-walled non-laminar clean rooms to determine whether this was a possible alternative to laminar flow rooms [27, 30]. Two particular enclosures at General Electric were examined; enclosure A is 13.7 by 4.6 meters (45 by 15 feet), with air flow of 3 to 4.5 meters (10 to 15 feet) per minute, and enclosure B is 3.7 by 4.25 by 2.4 meters (12 by 14 by 8 feet) with an air flow of 15.25 meters (50 feet) per minute. They found that enclosure A met the specifications of a class 5000 to 10 000 clean room and enclosure B met the specifications of a class 100 clean room. Both enclosures meet the microbial specifications of not more than 3.6 viable particles per cubic meter (0.1 viable particles per cubic foot) of air. They recommend that further studies be done toward a possible relaxation of the class 100 laminar flow room requirement.

Costs and efficiency will play a large part in the choice of assembly methods. NASA is presently in the process of examining various clean rooms to determine which ones are suitable or could be made suitable for bioclean assembly. It is recommended [27] that types of clean rooms other than rigid laminar downflow rooms be considered.

b. Monitoring. Careful monitoring is necessary to insure that low microbial levels are maintained and specified procedural precautions are strictly adhered to.

In Jet Propulsion Laboratory's Experimental Assembly and Sterilization Laboratory (EASL) [31], a Royco particle counter was used to monitor particulate air contamination, a Reniers slit sampler was used for microbial air sampling, stainless steel settling strips were used for measuring microbial fallout and procedural monitoring was done by visual and camera surveillance. They report that the steel strips were very satisfactory, but that both air monitoring methods had the disadvantage of taking such small samples that it was questionable whether they could be said to be representative of the room air. Particulate monitoring methods have been investigated by the Martin Company [32] and others [33,34], and the microbial monitoring is continually being studied (see discussion of biological monitoring in the third section of this report).

c. Personnel. It is agreed that microbial contamination of clean rooms is a function of the number and activity of the personnel in the room [35]. Much research has been and is being done, and all aspects of personnel activities, dress, entrance and egress, health and monitoring are being investigated. A detailed recapitulation of the information obtained is beyond the scope of this report. The most comprehensive and complete information can be found in the General Electric Final Report [27], Section 11. It is evident that extensive and costly modifications of conventional clean room operations are necessary.

d. Existing Facilities. Jet Propulsion Laboratory has built an Experimental Assembly and Sterilization Laboratory (EASL). The facility is based on (1) laminar downflow, (2) small positive pressure gradient, (3) decontamination of all parts, etc., entering the facility and (4) rigorous personnel cleanliness techniques. The floor plan, showing sampling locations, is given in Figure 2. The objectives specified for the EASL effort were as follows:

1. To evaluate the new NASA "Interim Requirements" provision in terms of resulting microbial contamination in finished assemblies , quality and reliability of finished assemblies and time, human factor, and economic considerations.

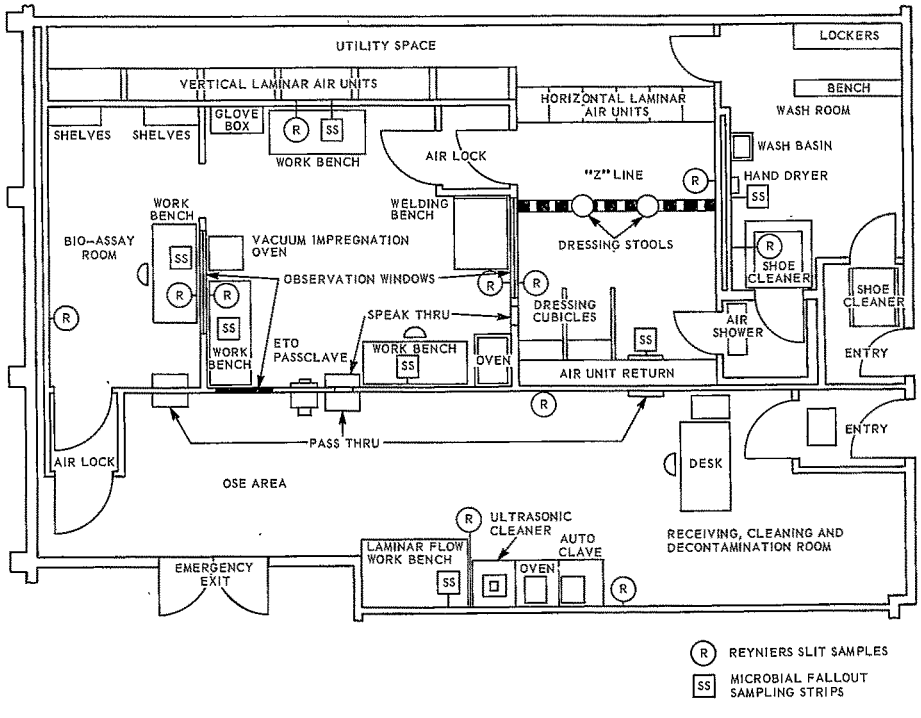


FIGURE 2. EASL FLOOR PLAN

2. To develop and obtain, for existing and future facilities, design and operational requirements, microbiological monitoring requirements and techniques for fabrication, assembly and test operations and quality assurance requirements and procedures.

A detailed report on this facility can be found in Drummond and Magistrale and Kapell, McDade and Gavin [19, 31]. Microbial and particulate levels achieved were well within the specifications of Fed. Std. 209, the Interim Requirements and the new NASA document. They report that the hardware assembled in EASL appears to have a very low level of contamination.

Daniel, Mann, Johnson and Mendenhall, under contract to Jet Propulsion Laboratory, completed in 1964 a study of an Assembly Test and Sterilization Facility (AT&SF) in which they examined all aspects of assembling and sterilizing a spacecraft [36]. They established the following guidelines:

1. The spacecraft assembly area will be in a clean room.

2. The material entering the clean room will be surface cleaned with ETO, pressurized steam or liquid decontamination agents.

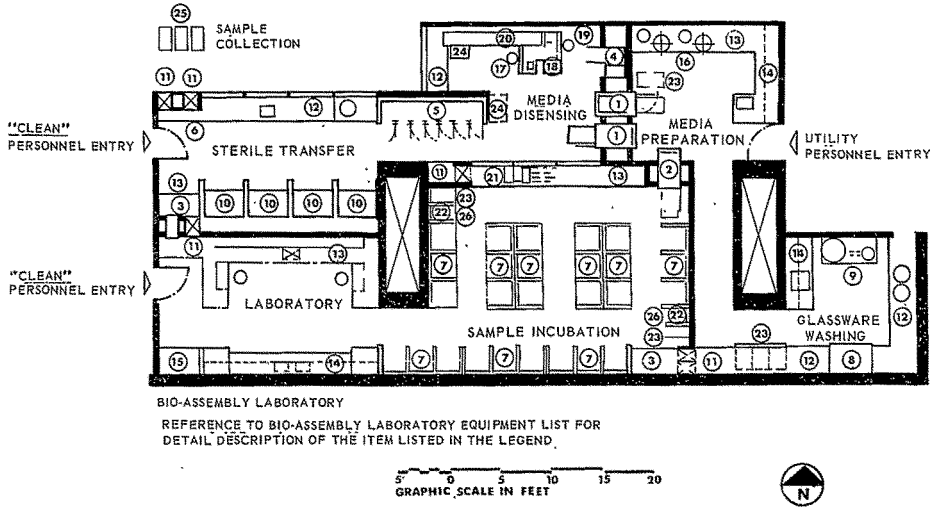
3. Microbiological monitoring will be conducted within the clean room.

4. The assembly will be protected from undue contamination by enclosing it in a biological barrier.

A salient aspect of the facility is the bioassay laboratory with a continuous biological assay plan. Figures 3 and 4 show the bioassay laboratory and petri dish flow plan which have been suggested [19].

They report that the design and construction of such a facility is feasible and can be constructed from on-the-shelf hardware at reasonable cost, and they estimate that a typical Mariner capsule would accumulate around 1.8×10^6 viable particles in this facility They recommend further development in the areas of automated microbiological assay laboratory devices, improved laboratory equipment, smaller and faster air particle counting, better techniques for microbial sampling and assay work, improved clean room clothing and headgear and additional functional analysis to determine human factors in man/machine interfaces in spacecraft sterilization technology.

In May 1966 Jet Propulsion Laboratory began work in a Sterilization and Assembly Development Laboratory (SADL). In this facility experience with a full-scale training capsule which is expected to provide necessary knowledge for the actual design of a sterilizable spacecraft will be obtained [7, 19, 36].



1 STERILIZER–STEAM VACUUM
2 STERILIZER–STEAM ETO COMBINATION
3 STERILIZER–ETO PASSCLAVE
4 PETRI DISH DRYING AND COOLING CABINET
5 PETRI DISH REFRIGERATOR
6 SAMPLER REFRIGERATOR (UNDER COUNTER)
7 SAMPLE INCUBATORS
8 LABORATORY GLASSWARE WASHER
9 WATERSTILL; 20 gph, 100 gal STORAGE
10 LAMINAR WORK STATIONS
11 AIR LOCKS (APPARATUS PASS THROUGH)
12 SS COUNTER
13 BASE CABINETS AND DRAWERS
14 WALL CABINETS
15 UTILITY STORAGE CABINET
16 MEDIA PREPARATION SYSTEMS
17 LAMINAR DISPENSING STATIONS
18 TRAY FEED MAGAZINE
19 AUTOMATIC PIPETTERS
20 TRAY CONVEYOR
21 PETRI DISH MARKING AND SORTING UNIT
22 AUTOMATIC PETRI DISH SCANNER
23 UTILITY CARTS
24 TRAY TRANSFER CARTS
25 SAMPLE COLLECTION CARTS
26 SCANNER CARTS

FIGURE 3. AT&SF BIOASSAY LABORATORY FLOOR PLAN

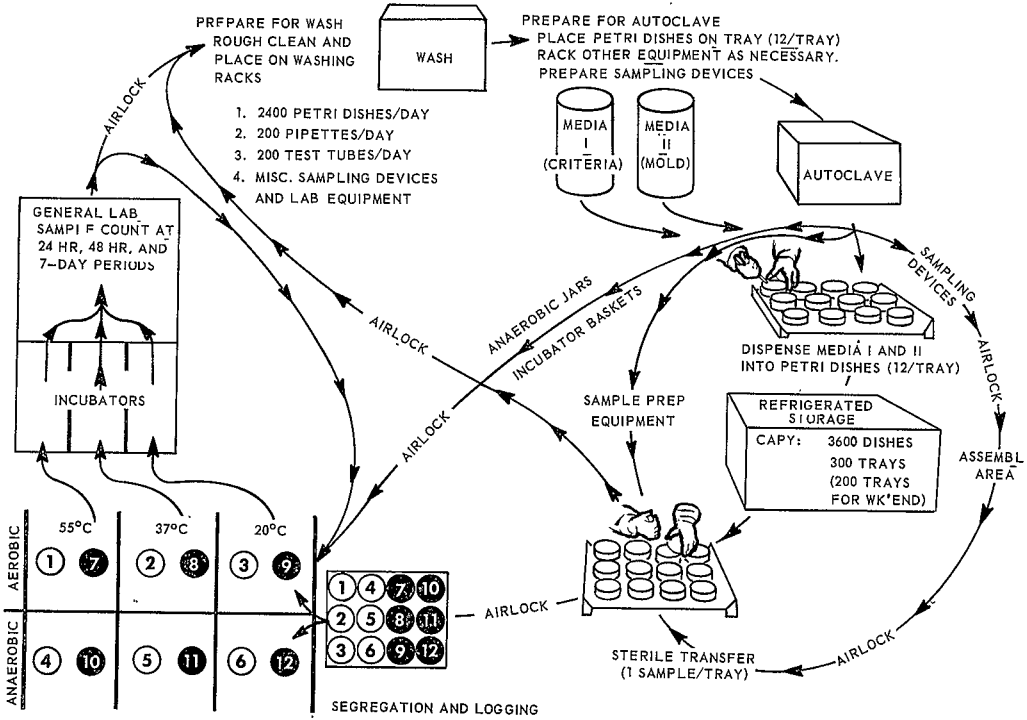


FIGURE 4. AT&SF BIOASSAY LABORATORY PETRI DISH FLOOR PLAN

General Electric, in conjunction with their pilot spacecraft assembly, has converted its controlled environment facility into a bioclean facility. This includes a class 100 laminar downflow room and a 67 square meter (722 square foot) bioassay laboratory [25].

A bioclean room has recently been installed at Goddard Space Flight Center in Greenbelt, Maryland [37].

PLANETARY MISSION PLANNING

Introduction

The general plan for the sterilization of unmanned planetary landers is as follows [22]:

1. Develop sterilizable capsule hardware.

2. Limit the quantity of viable biological loading.

3. Apply terminal sterilization.

4. Protect the sterile capsule from recontamination through launch and and until impact on the planet. (Nominal assembly and sterilization procedures for the Voyager capsule are shown in Figure 5 [7])

The following paragraphs present a summary of the present status of development in each of these phases, with special emphasis on the problems involved in each phase.

Sterilization Compatibility and Reliability

With the requirement for a sterile Mars landing, sterility joins reliability as top priority criteria governing the development of the entire mission. These two considerations are not necessarily opposing, but extremely careful planning is required to insure the achievement of both, since heating tends to reduce reliability. This involves both the capsule design and the materials used.

1. Design. - The requirement for sterility must be considered in the design of the capsule. In designing the configuration of the capsule, areas and crevices which may collect contamination should be minimized. The need for

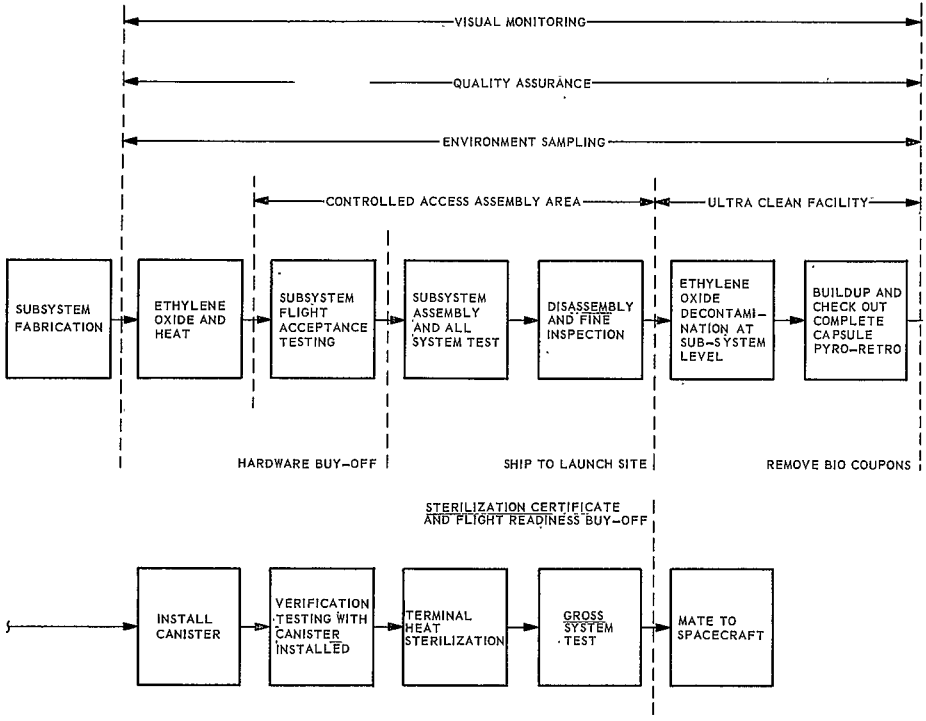


FIGURE 5. NOMINAL ASSEMBLY AND STERILIZATION PROCEDURES, VOYAGER CAPSULE

a clean design is consistent with low particulate contamination and, therefore, reliability. A major source of particulate contamination is provided during last minute design modifications at the launch site which require machining such as drilling. General Electric has examined some of the causes of machine reworking. They recommend that sufficient time should be allotted in schedules to permit changes to be integrated into the vehicle before it enters the highly controlled final assembly area, and that a modular concept be used for equipment packaging such that standardized mountings are used for package installation and exchange or redesign of equipment requires minimum "dirty" machining [13].

The thermal properties of the vehicle with respect to a terminal heat sterilization cycle must also be considered. Heat sterilization is accompanied by two major effects which bear design consideration. These are the effects of transient thermal gradients during heat-up and cool-down, and structural stresses due to high temperatures.

The effects of transient thermal gradients are dependent on the rate of heating or cooling, the method of heat application and the magnitude of thermal resistance paths [38]. Several analyses have been done on similated capsules to study heating and cooling phenomena [5, 13, 38, 39]. They show that thermal sterilization is an important design criteria. The most recent one reports that for a 907-kilogram (2000-pound) lander it may take as long as 400 or 500 hours for the innermost parts to reach the temperature of the surface [5]. This reference reports that the compensating effects of slower heat-up and cool-down of internal components essentially equalizes the accumulated sterilization, the maximum variation being 10 percent for three locations in any one configuration. Presently approved sterilization cycles are based on the assumption that the capsule heats and cools instantaneously. For a Voyager capsule of this expected size, it appears that this assumption is impractical. Schalhowsky [5] recommends the use of a suitable time-temperature integral over a range of temperatures, which could be implemented by time varying oven cycles so that required sterilization with its accompanying deleterious effects would not be greatly exceeded anywhere. Thus, the thermal conducting properties of materials used must be considered in design.

Structural analyses shows that thermal sterilization is an important criterion, particularly in pressure vessels and tanks, pyrotechnics and multilayer structures. Analyses on spherical, toroidal and cylindrical liquid or gas vessels show that the increased pressure of gases caused by high temperature and a 20 percent material degradation allowance imposes a relatively high weight penalty (on the order of an 80 weight increase [9]) on tanks which are charged before final sterilization [13].

In summary, some desirable design features of a sterilizable lander as listed by General Electric [27] are:

1. simple geometry for ease of cleaning

2. design of joints for high thermal conductivity

3. avoidance of large concentrated thermal masses

4. good thermal conductance between heat shield and structure

5. avoidance of sharp transition from thin to thick structure numbers.

6. reduction in the number of alignment sensitive components and care in supporting them from major structural components subject to small thermal gradients

7. special consideration for tanks and pressure vessels because of either weight penalties or need for sterile disconnects

8. due regard for the interface problems posed by the need for a biological barrier (canister).

2. Parts and Materials. In present spacecraft technology, many of the materials and components are incompatible with the sterilizing conditions In general, the ETO-F12 mixture is more compatible with spacecraft components than heat; however, it has been pointed out that any chemical applications are natural enemies to reliability, and that compared to the large store of thermal data, there are relatively few accumulated data on ERO mixture effects. Compatibility tests to date, however, show that most parts are unaffected by the mixture. Any irreversible effects on the reliability of electronic components appear to be caused by the water vapor in the mixture, which has long been known as an enemy to reliability [40].

Heat sterilization is destructive to the material and functioning of many of the parts currently being used in the spacecraft industry. Besides the hope of future development of sterile insertion techniques, there are two alternatives for overcoming this obstacle. The first and major one is the development of components and materials which can withstand the prolonged heating during sterilization; the second is the protection of heat sensitive components previously sterilized by other methods.

a. Heat sterilizable components and materials. The main emphasis is on the development of parts and materials which can withstand heat sterilization. In general the most reliable parts are the most likely to be sterilizable and the sterilizable components are the most likely to be reliable [40]. Developers of heat sterilizable components and materials report that the end goal of a totally heat sterilizable lander is feasible.

Jet Propulsion Laboratory requires that items acceptable to be sterilized by heating for a planetary lander must meet the following specifications [19, 41]:

1. Heat Sterilization: Type approval test (nonflight 145°C for 36 hours, 3 cycles) (flight acceptance tests to provide sterility, 135°C for 24 hours, 1 cycle equipment).

2. Ethylene Oxide Decontamination: Type approval tests 12 percent Ethylene Oxide, 88 percent Freon-12, 24 hours at 25°C and 24 hours at 40°C for two cycles. (40 to 50 percent relative humidity).

Jet Propulsion Laboratory is currently involved in a major program to establish an approved list of heat sterilizable electronic components and do research on all aspects of high temperature, longevity of electronic parts, etc. [19]. The present JPL program phase involves 42, 814 parts made up of 262 part types, and will produce 418 000 000 part-test hours of data. The tests include several groups of parts undergoing 10 000-hour life testing during and/or after varying numbers of sterilization cycles. This phase of the program will be completed in 1967, and a follow up program is planned for the replacement of unapproved parts.

Work is also being done by JPL and subcontractors on polymeric materials and scientific instruments, two of the biggest problem areas. Polymeric materials are the least likely to be internally sterilized by their manufacturing processes and are the most difficult to assay (see previous discussion of assay techniques). The greatest difficulty, however, is the development of scientific instruments which can be sterilized by heating. Developments in the area have met with only limited success. Some problem components are sampling devices, plastic seals, lubricants, electrodes, dialysis membranes, chemical reagents and biological media; many of these are inherently heat labile, and sterilization of these may have to depend on methods other than heat, followed by sterile insertion or protection from heat during capsule sterilization [19].

b. Protection of Heat Sensitive Components. The feasibility of protecting heat sensitive internal components during sterilization is under study by G. E. [13] under contract to NASA. In particular, their work covers one passive method, insulation, and one active one, the use of thermoelectric cooling devices.

Insulation provides a thermal lag for the component, thus the shortest approved sterilization cycle, 160 C for three hours, was used for the study. An analysis was done on 7.62-, 10.16- and 15.24-centimeter (3-, 4-, and 6-inch) cubes having 2.54, 5.08 and 7.62 centimeters (1-, 2-, and 3-inches) of insulation of several thermal diffusivities. They found that the temperature reached by the 15.24-centimeter (6-inch) component with 7.62 centimeters (3-inches) of the best insulator was the lowest, but that all components reached at least 140°C. The weight penalty was of the order of 0.9072 kilograms (2-pounds) for a 10.16-centimeter (4-inch) cube with 5.08 centimeters (2-inches) of insulation.

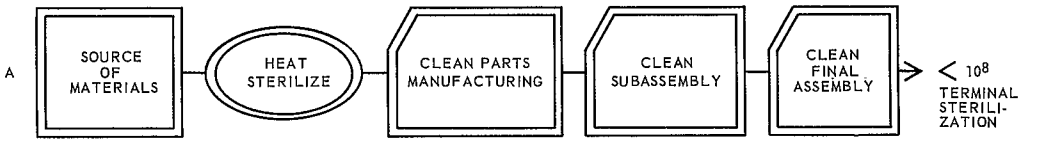
Thermoelectric cooling modules take advantage of the temperature difference, due to the Peltier effect, which is maintained when direct electrical current is passed through the junction of two dissimilar conductors. (The same principle can be used in heating.) This allows for steady-state operation with the component at a lower temperature. Factors limiting the use of such devices are the difficulty of heat removal to the sterilization environment and the fact that thermoelectric modules have a maximum allowable hot-side temperature. A theoretical analysis was made of four available modules using 105°C to maintain a component at 71°C. None of these modules was equal to the task.

Reduction of Bioload Previous to Terminal Sterilization

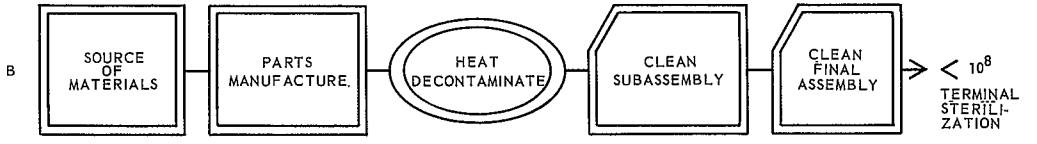
1. Introduction. The terminal sterilization cycles are based on a presterilization load of not more than 10^8 organisms per vehicle. Extensive work is currently underway to achieve this with the highest mission reliability and the lowest program cost. When methods of monitoring, assaying and decontamination become sufficiently refined, it is hoped that the terminal sterilization cycles can be reduced, and thus the problems due to the prolonged heating of the lander system would be cut down.

There are three general approaches, which are shown in Figure 6 [22]. The first permits only one heating and requires bioclean assembly

CLEAN HANDLING THROUGHOUT



HEAT DECONTAMINATION OF PARTS, CLEAN SUBASSEMBLY



HEAT DECONTAMINATION BEFORE CLEAN FINAL ASSEMBLY

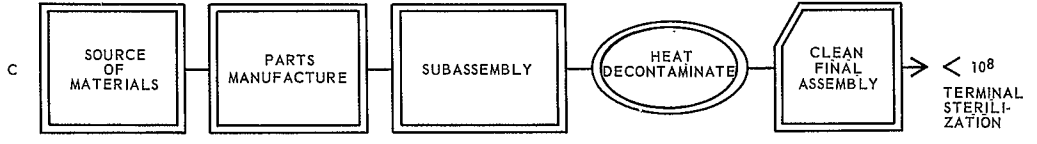


FIGURE 6. LIMITATION OF BIOLOGICAL LOADING

throughout. The second allows normal assembly, heat decontamination of assayable parts and subsequent clean assembly. The third allows normal manufacturing of subassemblies to a nonassayable size, a "worst case" decontamination cycle and subsequent clean assembly. Monitoring of procedures by visual means, air sampling and biological fallout strips will be required in all clean assembly steps. Reliability factors will probably determine the final choice [22].

2. Piece Parts. G. E. has recently completed an experimental evaluation of detailed manufacturing procedures and parts procurement [27]. In general it is recommended that piece parts be manufactured under normal conditions and then sterilized or decontaminated before incorporation into subassemblies. Many parts manufacturing procedures involve heat curing cycles such that with only minor modifications they can be altered to yield internally sterile parts.

3. Subsystems.

a. Procurement. Prime contractors obtain much of their hardware from subcontractors. On a recent program General Electric reports [27] that subcontracting represented 60 percent of total hardware cost and 35 percent of hardware items. This means that larger items were bought, and smaller items were made by the prime contractor. This presents the problem of educating suppliers of major subassemblies in bioclean assembly techniques. General Electric has conducted surveys to evaluate vendor requirements, and they find that this may be a major problem.

NASA is currently sponsoring a series of training courses for professional technical workers to familiarize them with microbiology. The course is entitled "Environmental Microbiology for Engineers," and the University of Minnesota is giving the course.

b. Assembly. Starting at the subsystem level, according to the Voyager Quarantine Plan [7] the various developmental contractors will be

required to assemble hardware in a certified Planetary Quarantine Clean Assembly Facility, with appropriate monitoring. In studying actual assembly procedure in their pilot spacecraft assembly, however, G.E. has compared various cleaning and decontamination techniques, and found that comparable low levels of biological contamination can be achieved in many cases, without the use of expensive clean rooms, by incorporating minor changes into the standard assembly procedures. General Electric has issued, in conjunction with the final report on this phase of their contract [27], a Procedures Manual Volume II, Manufacturing Procedures, [14] which details these procedures.

Sterile assembly as a practical alternative to total heat sterilization was experimentally evaluated by Lockheed [26]. They carried out the assembly of a small electronics unit in a glove box under sterile atmospheres of nitrogen, Ethylene Oxide-Freon 12 and air. They found that maintenance of sterility throughout aseptic assembly is feasible, but that the effectiveness of ETO in sterilizing some of the typical components was not demonstrated.

In general, although specifications require assembly in clean rooms to maintain a reduced bioload, this involves a large time, cost and personnel penalty. G.E. recommends the use of normal aerospace conditions whenever possible, followed by decontamination. This trend is also evidenced in a paper from AVCO [42] in which a method of calculating total bioload for analytical comparison of assembly techniques is presented. They report that required levels of biological contamination can be achieved without the requirement that parts and components to be delivered to the capsule system assembly contractor be produced under other than normal conditions.

4. Packaging and Storage. Storage of nonclean assembled parts at elevated temperatures for a relatively long period has been considered for bioload reduction [27]. For example, storage at 105°C for two weeks (336 hours) is the same as one of the sterilization cycles. This would constitute decontamination, rather than sterilization, since it is not assumed that the original bioload is particularly low.

Packaging materials for sterility and biocleanliness maintenance are being investigated [27]. Present conclusions indicate that due to the many properties necessary--sterilizability, cleanability, slough resistance, heat sealability, transparency and permeability only to ETO--a combination of materials will probably be used.

5. Final Assembly. Final assembly before sterilization will be done in a Class 100 vertical laminar downflow cleanroom [7, 22, 27, and 29].

Launch Site Flow and Terminal Sterilization

1. Introduction. Since the final assembly is to result in a relatively low level of biological contamination, it is recommended [27] that the lander be transported to the launch site sealed in its canister which is in turn sealed inside a shipping container. A positive pressure inside the canister is to be maintained and can be monitored to insure the integrity of the canister as a bacteriological barrier. Upon arrival at the launch site, it will be inspected in a bioclean area, in order to maintain the low bioload.

Sterilization heat cycles have been discussed in Section II. Analytical studies have shown that heating by convection of an inert gas is more efficient than heating in a vacuum. The present state of the art requires that a terminal sterilization heat cycle is applied to the completely assembled lander inside its canister, which is then sealed.

The method of implementing the terminal launch-site sterilization cycle is being studied [27, 36]. Several typical launch site flow plans have been proposed; a specific one cannot be made until an actual mission is designed. Previous sterilization plans have been proposed by G. E. in 1963 [15] and Daniel, Mann, Johnson and Mendenhall under contract to JPL in 1964 [19].

2. Present Sterilization Plan. In conformance with the sterilization technology in 1965, the Voyager 1973 design has the following basic ground rules [27]:

1. bioclean assembly at the contractor's plant
2. terminal sterilization at the launch site
3. launch by Saturn V Booster.

Critical aspects of the launch site flow plan are:

a. Ordinance Installation. Due to the closed nature of the lander-canister system, a significant portion of measuring devices will have to be installed before systems test, because later installation would require disassembly of the vehicle, thereby negating the results of the systems test.

b. Launch Pad Compatibility Tests. It is recommended that launch site compatibility tests be performed either with a development vehicle or with a flight backup vehicle in order to eliminate one trip to and from the Vehicle Assembly Building, eliminate an extra mechanical and electrical mating and stage separation and eliminate a significant amount of time in the Vehicle Assembly Building.

This is significant from a contamination control viewpoint, too; the only disadvantage is that the test would not be on the specific vehicle. Since the vehicles are to be identical, however, this is not considered to be a serious disadvantage.

c. Saturn V Considerations. The goal of restraints from this consideration is to delay mating with the Saturn V as long as possible to minimize the time between sterilization of the lander and launch [27].

During the last time that the canister and lander are separated, the lander is to be bioassayed for the last time. G. E. has developed an approach for estimating the total bioload, based in their pilot spacecraft assembly. This approach involves the estimation of the total surface area and allows calculation of total bioload. The method also permits the allocation of contamination levels to various components during subassembly to achieve a specific final level, and permits extrapolation with respect to vehicle size.

d. Possible Future Sterilization Plan. Although no methods of sterile assembly have been approved by NASA to date (July 1966), the use of an Assembly/Sterilizer, shown in Figure 8, has been proposed by G. E. and is under investigation [43]. This facility provides for sterile operations on the spacecraft before and after sterilization. The decontaminated vehicle is placed in the facility, disassembled, sterilized and

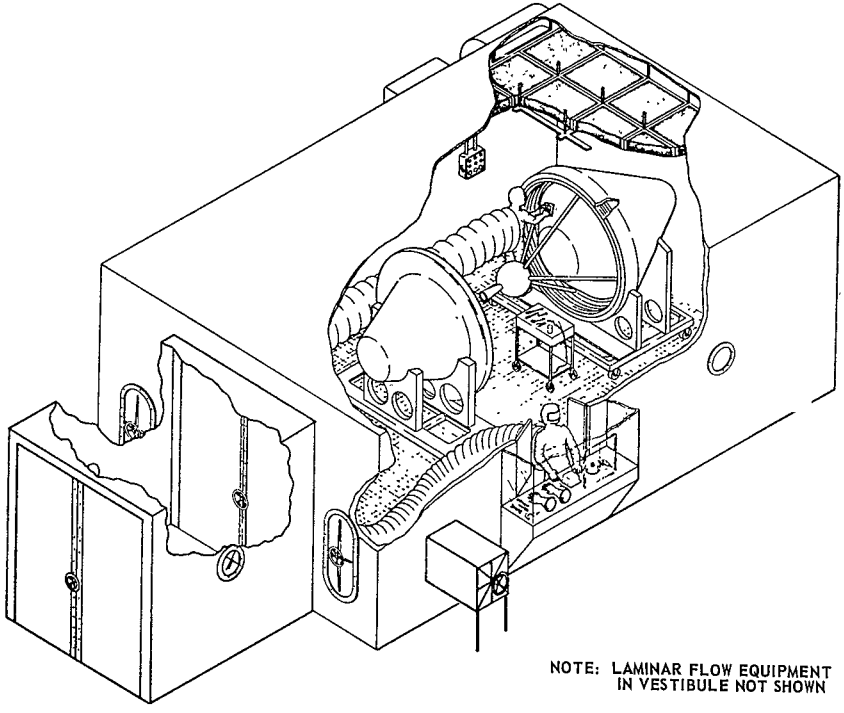


FIGURE 7. VISUAL CONCEPT OF MAIN STERILIZATION CHAMBER OF THE ASSEMBLY/STERILIZER

then reassembled by workers topologically separated from the sterile environment by bioisolator suits. Repairs can be made, or replacement parts can be brought in and sterilized in pass-through chambers. Another advantage would be the minimization of the thermal structural problems discussed in an earlier section by sterilization in a disassembled state and separate sterilization of liquids, gases and pyrotechnics. Use of this facility would allow for a simplified canister design and would allow placing a sterile bag over the canister. NASA has not yet (as of July 1966) approved any sterile assembly methods, but the feasibility of this facility is presently being investigated by General Electric under contract NAS1-5381 [27, 43, 44].

Sterility Maintenance After Terminal Sterilization

1. Before Launch. After sterilization, the lander is sealed in a biological barrier to maintain its sterility until impact on the planet. General design criteria for the canister are as follows [27]:

1. Keep out bacteria, spores, and other organisms.

2. Keep the canister as light as possible, since it accompanies the lander into space.

3. Be able to contain the capsule and any remote handling gear.

4. Incorporate sterile electrical connectors for flight, checkout, and test.

5. May act as a meteroid bumper if weight penalty can be tolerated, and if retained until just before planetary entry.

6. Contain sterile plumbing fittings for liquids and gases, if required.

7. Remain unaffected by hot or cold (Ethylene Oxide-Freon F12) sterilization methods.

8. Remain unaffected by dry heat up to 145° C.

9. Eject the capsule before planetary entry without compromising sterility.

The selection of materials, rigid or flexible, depends on the size and shape of the lander. It has been recommended [39] that rigid canisters be used for small landers and that a combination rigid and flexible structure be used for larger vehicles.

Prevention of canister penetration by microorganisms will be implemented by maintenance of a 6894 newton per square centimeter (1 psi) positive pressure inside the canister, so that any leaks will be outward. This will also provide an easy way to monitor the integrity of the canister.

The AVCO Corporation has recently completed a contract to design, fabricate and test a rigid container for a 76.2-centimeter (30-inch)-diameter sphere-cone atmospheric probe, and to evaluate its effectiveness as a biological barrier after exposure to qualification level sterilization heat cycles. Areas of interest in this study were barrier integrity, thermal gradients, thermal stresses, effects of different types of pressurizing gas and effects of free and forced convection heat transfer on the system [45]. They report that existing technology will enable the design and fabrication of such a canister.

2. After Launch. Detailed analysis is required to prevent the contamination of the lander after launch and contamination of the planet from accidental impact of contaminated parts of the launch vehicle or canister. Some tentative contamination probability assignments have been discussed in the second section of this report. Contamination sources include penetration of the canister during flight, recontamination from various sources at the time of lander deployment from the canister and accidental impact on the surface of lander or planet by spacecraft ejecta such as attitude control gas, material outgassing and propulsion system gasses. A more complete outline of possible sources of contamination can be found in JPL's Planetary Quarantine Plan [7].

The canister development contract by AVCO includes a study and analysis of deployment mechanisms with respect to contamination.

CONCLUSIONS AND RECOMMENDATIONS

It appears that present technology will be able to provide, as mission development requires, the capability to land reliably a sterile vehicle on another planet; the obstacles are tremendous, however, and a concentrated effort in all areas of planning, engineering manufacturing and assembly is required. Technology for the implementation of a planetary quarantine policy is expanding very rapidly around mission guidelines which are themselves in developmental stages. This presents the potential hazard that mission guidelines may be adopted that are not necessarily the best consistent with existing technology in terms of reliability, cost and overall mission objectives. An example of this is the possible rejection of the inflight sterilization concept because it was suggested after plans around the terminal heat sterilization concept were well underway. For optimum achievement of the goals of interplanetary missions, it is recommended that maximum flexibility in fundamental policy as well as all other planning phases be maintained.

REFERENCES

1. Anon.: Significant Achievements in Space Biosciences 1958-1964. NASA SP-92, 1966.

2. Anon.: NASA Unmanned Spacecraft, Decontamination Policy. NASA Management Manual, no. 4-4-1, ch. 4, Sept. 9, 1963.

3. Personal communication with Dr. Carl Bruch, NASA Headquarters, August 1966.

4. Sagan, C. and Coleman, S.: Spacecraft Sterilization Standards and Contamination of Mars. Aeronaut. Astron., May 1965.

5. Schalhowsky, Samuel (Principal investigator): Study of Analytical Techniques in Dry Heat Sterilization of Planetary Spacecraft. Exotech Inc., Third Monthly Progress Report, Jan. 20, 1966.

6. Light, J. O.: Discussion of Planetary Quarantine Constraints. Presented at COSPAR (Vienna), May 11-18, 1966.

7. Anon.: Planetary Quarantine Plan. JPL PQ 001 BB 001 1B51, Mar. 15, 1966.

8. Fisher, Pearl D.: Evaluation of the Feasibility of In-Flight Sterilization of Mars Probes. Presented at Stepping Stones to Mars AIAA Meeting (Baltimore, Maryland), March 29, 1966.

9. Anon.: Procedures Manual for Planetary Spacecraft to be Sterilized by Heating, Volume I, Design Guidelines. General Electric Co., July 31, 1965.

10. Opfell, J. B. and Newman, T. W.: A System Engineering Approach to Spacecraft Sterilization Requirements. Presented at Stepping Stones to Mars Meeting of AIAA (Baltimore), March 29, 1966.

11. Barrett, M. J. and Cooley, W. C.: On the Feasibility of Radiation Sterilization of Planetary Spacecraft. Exotech Inc., Final Report, NASA Contract NASW-1320, May 2, 1966.

REFERENCES (Cont'd)

12. Portner, D. M.; Spinner, D. R.; Hoffman, R. K.; and Phillips, C. R.: Effect of Ultrahigh Vacuum on Viability of Micro-organisms. Science, vol. 134, no. 3495, 1961, p. 2047.

13. Anon.: Design Criteria for Typical Mars Landing Capsules Requiring Sterilization. General Electric Co. First Quarterly Progress Report, Phase III, NASA Contract NAS8-11372, Apr. 1, 1966.

14. Anon.: Procedures Manual for Planetary Spacecraft to be Sterilized by Heating, Volume II, Manufacturing Procedures. General Electric Co., Preliminary, July 5, 1966.

15. Anon.: Voyager Design Study, Volume V Sterilization. General Electric Co., NASA Contract NASW-696, Oct. 15, 1963.

16. Anon.: Handbook for Contamination Control on the Apollo Program. General Electric Co. (Draft) ASD-QA-12-65-6, NASA Contract NASW-410, Dec. 1, 1965.

17. Anon.: Russian Sterilization Report. Contamination Control, American Association for Contamination Control, volume V, no. 7, July 1966.

18. Decker, H. M. and Buchanan, L. M.: Filtration in Spacecraft Sterilization. Presented at NASA First National Conference on Spacecraft Sterilization Technology (Pasadena, California), Nov. 16-18, 1965.

19. Drummond, D. and Magistrale V. (Compilers): JPL Spacecraft Sterilization Technology Program: A Progress Report. Jet Propulsion Laboratory TR-32-853, Dec. 31, 1965.

20. Opfell, John B. and Bandaruk, W.: Microbial Contaminants in the Interiors of Spacecraft Components. (Philco) COSPAR, Sixth International Space Science Symposium (Buenos Aires, Argentina), May 1965.

REFERENCES (Cont'd)

21. Kohorst, D. P. and Harvey, H.: Polymers for Sterilized Spacecraft. Presented at NASA First National Conference on Spacecraft Sterilization Technology (Pasadena, California), Nov. 16-18, 1965.

22. Hall, Lawrence B.: NASA Requirements for the Sterilization of Spacecraft. Presented at the First Annual National Conference on Spacecraft Sterilization (Pasadena, California), Nov. 16-18, 1965.

23. Memorandum from Dr. Reynolds to O. W. Nicks, Director of Lunar and Planetary Programs, May 26, 1966.

24. Anon.: Standard Procedures for the Microbiological Examination of Space Hardware. NASA Headquarters, Washington, D. C., June 1, 1966.

25. Anon.: A Proposal for a Handbook on the Biological Aspects of Spacecraft Sterilization. General Electric Co., Mar. 14, 1966.

26. Anon.: Experimental Study of Sterile Assembly Techniques. Lockheed Missile and Space Co., Final Report, Jet Propulsion Laboratory Contract No. JPL 950993.

27. Anon.: Development of Manufacturing Procedures for Planetary Spacecraft to be Sterilized by Heating, First (5 August 1965), Second (5 November 1965), and Third (5 March 1966) Quarterly Progress and Final Report, General Electric Co., Phase II NASA Contract NAS8-11372, July 19, 1966.

28. Anon.: Standard Bioclean Room and Work Station Requirements for the Microbiologically Controlled Environment. NASA Headquarters, Washington, D. C., Preliminary Draft. 1966.

29. Clean Room and Work Station Requirements, Controlled Envronment. Fed. Std. 209. Dec. 16, 1963.

REFERENCES (Cont'd)

30. Beyerle, F. J. and Lorsch, H. G.: Manufacturing and Handling Procedures for Planetary Spacecraft to be Sterilized by Heating. Presented at First Annual National Conference on Spacecraft Sterilization Technology (Pasadena, California), Nov. 16-18, 1965.

31. Kapell, G. F.; McDade, J. J.; and Gavin, T. R.: Experimental Assembly and Sterilization Laboratory (EASL) Operations: Phase I. Jet Propulsion Laboratory TR-32-941, Apr. 15, 1966.

32. Anthony, H. V.; Drew, J. G.; La Rue, E. F.; and McDonald, J. P.: Study of Contamination Sensors. Volume II, Final Technical Report, NASA Contract NAS10-2692, Martin-Marietta Co., Apr. 1966.

33. Anon.: Fabrication of Particle Counters for Clean Rooms. IITRI, Final Report for NASA Contract NAS8-20531, July 11, 1966.

34. Anon.: Particulate Matter Challenge for a Validation of the Maintenance of Sterility within a Sterile Assembly System. McDonnell Aircraft Corp., Unsolicited Proposal, Jan. 17, 1966.

35. Magistrale, V. J.: Engineering Problems in Capsule Sterilization. Astronaut. Aeron., vol. 4, no. 2, February 1966.

36. Daniel; Mann; Johnson; and Mendenhall: Preliminary Engineering Report For Sterile Assembly Development Laboratory. Performed for Jet Propulsion Laboratory, June 1965.

37. Anon.: Standard Operating Procedures - Experimental Clean Assembly and Sterilization Laboratory. Melpar, NASA Contract NAS5-8718, July 1966.

38. Tenney, J. B., Jr.; Fried, E.; and Crawford R. G.: Thermal Sterilization of Spacecraft Structures. Presented at AIAA Unmanned Spacecraft Meeting (Los Angeles, California), Mar. 1-4, 1965.

REFERENCES (Concluded)

39. Anon.: Design Criteria for Typical Planetary Spacecraft to be Sterilized by Heating. Final Report, Phase I, vol. I, General Electric Co., NASA Contract No. NAS8-11372, Dec. 15, 1965.

40. Bartholomew, C. S. and Porter D. C.: Reliability and Sterilization. Boeing Co., Presented at Stepping Stones to Mars Meeting of AIAA, (Baltimore), Mar. 29, 1966.

41. Anon.: Environmental Specification Voyager Capsule Flight Equipment and Flight Acceptance Test Procedures for the Heat Sterilization and Ethylene Oxide Decontamination Environments. JPL Spec. vol. 50503-ETS, Jan. 12, 1966.

42. Botan, E.; Gautrand, J. A.; Rider, T. and Schafer, W. J.: Biological Burden Estimation of Mars Probes and Capsules and a Method of Burden Control. AVCO, Presented at Stepping Stones to Mars Meeting of AIAA (Baltimore) Mar. 29, 1966.

43. Anon.: A Program to Demonstrate the Feasibility of an Assembly/Sterilizer Facility. General Electric, Unsolicited Proposal submitted to NASA Headquarters. Nov. 6, 1964.

44. Crawford, J. G. and Zanks, J. F.: The Assembly/Sterilizer - A Facility for the Sterilization and Assembly of Spacecraft. G. E., Presented at Stepping Stones to Mars Meeting of AIAA (Baltimore), March 29, 1966.

45. Anon.: Development of a Typical Mars Landing Capsule Sterilization Container. Final Report, AVCO Corp., NASA Contract No. NAS8-20502, June 28, 1966.

BIBLIOGRAPHY

1. Opfell; Miller; Kovar; Nutin; and Allen: Sterilization Handbook. Final Report, Dynamic Science Corp., NASA Contract No. NASW-777, Aug. 26, 1964.

2. Anon.: A Study of Design Guideline for Sterilization of Spacecraft Structure. Final Report, General Electric Co., NASA Contract No. NAS8-11107, Aug. 1964.

3. Thomas, Fred W., Jr. and Bergson, Myron H.: Maintaining Environmental Control Requirements for Fabrication and Assembly of Sterile Space Vehicles. General Electric Co., Institute of Environment Sciences 1965 Annual Technical Meeting Proceedings.

4. Nowitsky, A. M.: Spacecraft Sterilization Techniques and Equipment. Johnson Publishing Company, 1965.

5. Michaelson, G. B.: The Bacteriology of Clean Rooms. Progress Report WSG-643, University of Minnesota, Minneapolis, Minnesota, 1, Oct. 1964 - Mar. 31, 1965.

6. Nicks, O. W. and Miles, J. R.: Some Developments in Sterilization for Planetary Probes. Presented to COSPAR (Buenos Aires, Argentina), May 19, 1965.

7. Portner, D. M.: Dry Heat Sterilization of Micro-organisms at 105° F. Protection Branch Report of Test No. 19-65, Fort Detrick, Md, June 7, 1965.

8. Montgomery, L. C. and Marsh, H. E., Jr.: Sterilized Solid-Propellant Rocket Motors for Mars Landing Missions. Revision 1, Jet Propulsion Laboratory TR 32-725, June 30, 1965.

9. Tenney, J. B., Jr. and Crawford, R. G.: Design Requirements for the Sterilization Containers of Planetary Landers. AIAA paper 65-387, Presented at AIAA Second Annual Meeting, July 26-29, 1965.

BIBLIOGRAPHY (Cont'd)

10. Anon.: Standards and Guidelines for the Design and Operation of Clean Rooms and Clean Work Stations. U. S. Air Force Technical Order T. O. 00-25-203, Aug. 31, 1965.

11. Edelstein, L. and LeVora, N. W.: Sterilization of Spacecraft and Equipment. Martin Co., Presented at American Ordinance Association Seminar on Aerospace Packaging (Aberdeen Proving Ground), Sept. 29, 1965.

12. Powers, E. M.: Microbical Profile of Laminar Flow Clean Room. Goddard Space Flight Center, X-600-65-308, Sept. 1965.

13. Keister, F. Z.: Determination of the Effects of a Thermal Sterilization Process on the Mechanical and Electrical Properties of Soldered and Welded Joint. Hughes Final Report on Contract No. 951069 for Jet Propulsion Laboratory, subcontracted under NASA Contract No. NAS7-100, Oct. 9, 1965.

14. Terry, John E.: Sterilization of Unmanned Planetary Spacecraft. Document RSIC-490, Nov. 15, 1965.

15. Proceedings, NASA National Conference on Spacecraft Sterilization Technology (Pasadena, California), Nov. 16-18, 1965, (in press).

16. Quarterly Status Report. U. S. Army Biological Center (Provisional) Fort Detrick, Maryland, NASA Contract No. R-35, Nov. 1, 1965 - Feb. 1, 1966.

17. Life in Extraterrestrial Environments. Quarterly Status Report IITRI, NASA Contract No. NASr-22, Feb. 15, 1966.

18. Sterilization. A selected Bibliography from the Literature Retrieval System, Space Biology Branch, Goddard Space Flight Center Report X-450-66-53, Mar. 1966, (latest reference - Jan. 1965).

BIBLIOGRAPHY (Concluded)

19. Hall, Lawrence B.: Sterilizing Space Probes. International Science and Technology, Apr. 1966.

20. Stambler, Irwin: Mariner/Voyager: A New Fix on Mars. Space/Aeronautics, May 1966.

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